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FURTHER OBSERVATIONS ON THE EFFECTS OF ALCOHOL ON WHITE MICE¹

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IN a former paper ('11) it was found that white mice were not markedly affected when given alcohol in their food. Since this paper appeared Stockard ('12, '13, '16) has brought forth some striking and conclusive results demonstrating that guinea pigs are very sensitive to alcohol and decidedly injured by it. He administered the alcohol to his animals through the lungs by placing them in a tank containing alcohol at the bottom so that they had to inhale the fumes. His work raised the question as to whether similar results might be obtained with white mice by using the same method. Therefore it was decided to repeat my experiments, using the inhalation method.

For these experiments white mice eleven weeks old were obtained. They were all from one strain inbred to the fourth generation, two entirely distinct strains having been united to form this strain. They were divided into four lines, viz., a control line, a double alcohol line, that is, both males and females were subjected to alcohol, a female alcohol line and a male alcohol line. There were three cages in each line, each cage contained two females and one male, thus making six females and three males in each line. Two cages were made up of second generation alcoholized mice; both males were from the male alcohol line, two females from the same line and one female from the female alcohol line. The same cages were used as in my former experiments. They were made of 8-mesh wire and were 6 inches wide, 6 inches deep and 12 inches long.

The mice were kept in a laboratory room heated by steam. It was attempted to keep the room at a uniform temperature, but fluctuations occurred.

¹From the Laboratory of Physiology in the University of Oklahoma. For a review of the literature see Nice ('11 and '12). Also Stockard ('12, '13 and '16).

All the animals were fed the same food, consisting of wheat and kaffir corn with bread and milk once a day.

Every day except Sunday the double alcohol line, parents and young, the males of the male alcohol line, the females and young of the female alcohol line and the second generation of alcohol mice with their young were placed in a galvanized tank 26 inches long, 20 inches wide and 14 inches deep. Alcohol had been poured on to cotton which was placed under a wire mesh situated about 2 inches from the bottom of the tank, so the mice had to breathe the fumes. The mice were kept in the tank each day until they became intoxicated, as shown by their staggering gait or inability to stand up. At the beginning of the experiment the time necessary to intoxicate them was about one hour. Later they would often be kept in the tank for two hours. This shows that the mice acquired a tolerance for alcohol. This tolerance was shown after they had been treated about a month. To make sure that the mice were being heavily alcoholized, a few times they were left in the tank so long that they would not recover from the effects for three or more hours, and in some cases they did not recover from the intoxication, but died.

At the beginning of the experiment when the mice were placed in the alcohol tank they would sneeze, their eyes water and they would rush about in their cages, showing great uneasiness. Later they ceased to be so much disturbed, yet during the course of the experiment there was no indication that the mice liked the alcohol fumes.

THE WEIGHT OF THE ADULT MICE

The adult mice were weighed at the beginning of the experiment and once each month thereafter, to get an indication as to their health. Since they were nearly the same age and closely related, their average weights would be expected to be about the same unless the alcohol treatment had an injurious effect on them. By referring to Table X, it will be seen that there is only a slight difference between the various lines. The average weight

of the mice not treated with alcohol was 22.4 grams and of those treated with alcohol 21.8 grams.

TABLE I
AVERAGE GAINS OF ADULT MICE
Those Not Given Alcohol

Line	Sex	No. of Mice Weighed	Average Gain in Grams at 4 Months	No. of Mice Weighed	Average Gain in Grams at 7 Months
Control.....	Male	3	4.6	3	4.9
Control.....	Female	6	4.3	6	9.3
Male alcohol.....	Female	6	4.3	6	6.4
Female alcohol.....	Male	3	5.8		
Average gain of all mice not given alcohol.....		18	4.6	15	7.2

TABLE II
AVERAGE GAINS OF ADULT MICE
Those Treated with Alcohol

Line	Sex	No. of Mice Weighed	Average Gain in Grams at 4 Months	No. of Mice Weighed	Average Gain in Grams at 7 Months
Double alcohol.....	Male	3	3.2	1	4.0
Double alcohol.....	Female	8	4.8	2	4.2
Male alcohol.....	Male	3	3.0	3	3.5
Female alcohol.....	Female	4	3.5		
Average gain of all mice given alcohol.....		18	3.9	6	3.8

Tables I and II give the average gain of the different lines for four months and for seven months. The mice that did not receive alcohol gained more than those that were treated; the former gaining 4.6 grams on an average for four months and the latter 3.9 grams; in seven months the untreated mice gained 7.2 grams and the alcoholized 3.8 grams.

It is possible that the handling of the mice and the extra exercise they took in the excitement of being alcoholized might account in part for their growing less than the untreated mice. In the 1911 experiments the control mice carried 7 months gained only 2 grams on an average while the alcohol mice gained 6 grams; in the second generation carried four months the controls gained 1 gram each and

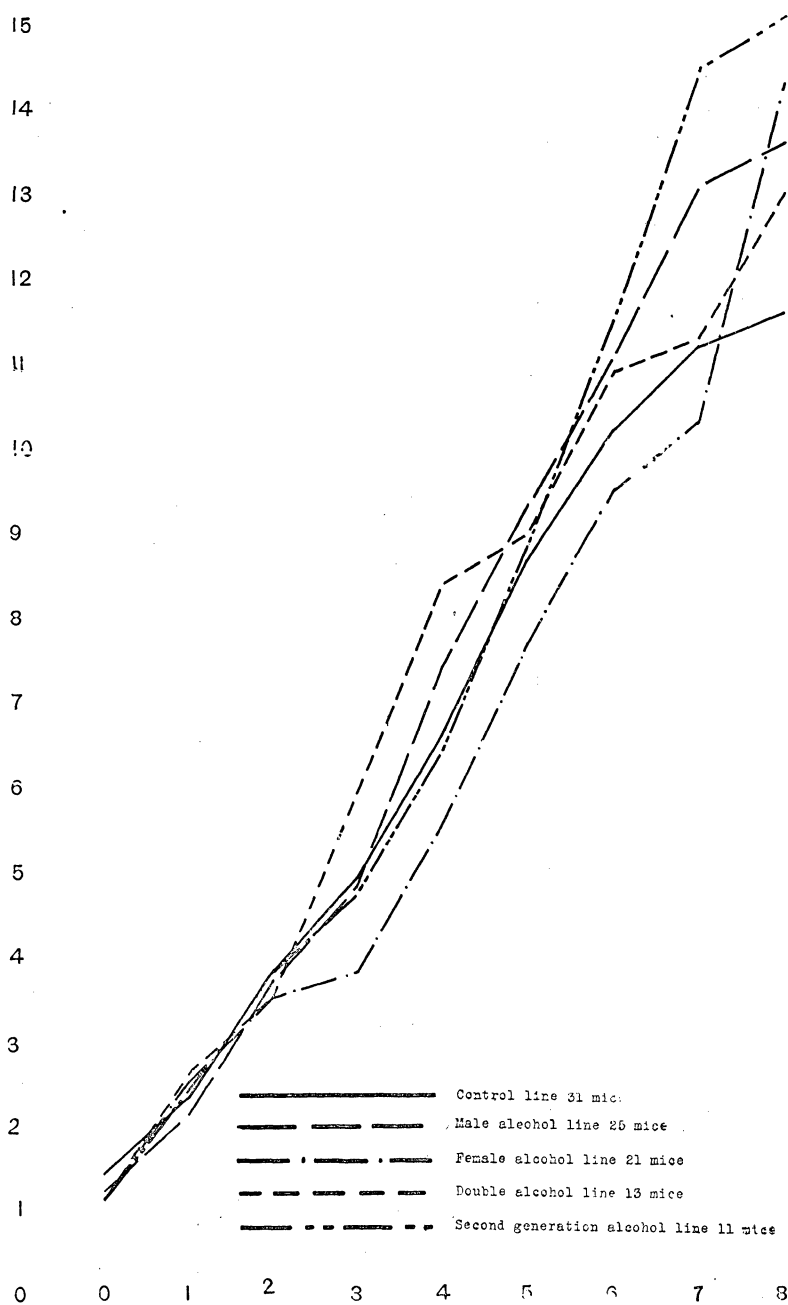


FIG. 1. Curve showing the growth of the mice. The abscissas represent the age of the mice in weeks and the ordinates their weights in grams.

the alcohol mice 2 grams. In that case the alcohol was given in the food and water and apparently had a fattening effect. None of those animals were handled except for weighing them.

RECORDS OF THE YOUNG OF EACH FEMALE

A record was kept of the young of each female. Many of the young in each of the lines were eaten by their parents. Tables III to VIII show the number of months each female was carried; the number of litters each had; the total number of young born, and the number that died apparently from lack of vitality.

TABLE III

RECORD OF THE YOUNG OF EACH FEMALE CONTROL LINE

Female	No. of Mo. Observed	No. of Litters	No. of Young Born	No. of Young That Died
<i>A</i>	7	1	7	0
<i>B</i>	7	2	10	0
<i>C</i>	7	1	6	0
<i>D</i>	7	1	5	0
<i>E</i>	7	1	6	0
<i>F</i>	7	1	2	0
<i>G</i> *.....	4	1	6	0
<i>H</i> *.....	3	1	5	0
Total.....	6:7 1:4 1:3	9	47	0

* Females *G* and *H* are second generation controls.

TABLE IV

MALE ALCOHOL LINE

Female	No. of Mo. Observed	No. of Litters	No. of Young Born	No. of Young That Died
<i>A</i>	7	2	12	1
<i>B</i>	7	1	12	0
<i>C</i>	7	2	10	0
<i>D</i>	7	1	3	3
<i>E</i>	7	1	5	0
<i>F</i>	7	3	24	0
Total.....	7	10	66	4

TABLE V
FEMALE ALCOHOL LINE

Female	No. of Mo. Observed	No. of Litters	No. of Young Born	No. of Young That Died
<i>A</i>	4	1	7	0
<i>B</i>	4	1	7	0
<i>C</i>	4	2	12	3
<i>D</i>	4	2	15	1
<i>E</i>	2	0	0	0
<i>F</i>	2	0	0	0
Total.....	4 : 4 2 : 2	6	41	4

Note.—Females *A*, *B*, *C*, and *D* were killed by being left in the alcohol tank too long. Females *E* and *F* were killed by accident.

TABLE VI
DOUBLE ALCOHOL LINE

Female	No. of Mo. Observed	No. of Litters	No. of Young Born	No. of Young That Died
<i>A</i>	4	1	5	0
<i>B</i>	4	1	7	0
<i>C</i>	9	0	0	0
<i>D</i>	9	3	20	0
<i>E</i>	4	2	15	2
<i>F</i>	4	2	15	0
<i>G</i>	4	1	5	1
<i>H</i>	4	1	6	2
Total.....	6 : 4 2 : 9	11	73	5

* These mice from *A* to *F* were left in the alcohol tank so long one day that only *C* and *D* survived the experience. After this accident one more cage, *G* and *H*, were made up from the original stock.

TABLE VII
SECOND GENERATION ALCOHOL LINE

Female	No. of Mo. Observed	No. of Litters	No. of Young Born	No. of Young That Died
<i>A</i>	2½	1	6	1
<i>B</i>	2½	1	10	0
<i>C</i>	2½	1	9	0
Total 3.....	2½	3	25	1

VIABILITY OF THE YOUNG

Table VIII shows the number of litters and the number of young born in each line; also the number that died from lack of vitality.

TABLE VIII
RECORD OF THE YOUNG OF EACH LINE
Summary of Tables III to VII

Line	No. of Mice	No. of Months	No. of Litters	No. of Young	No. That Died	Per Cent. That Died
Control	$\left\{ \begin{array}{l} 6 \\ 1 \\ 1 \end{array} \right.$	$\left\{ \begin{array}{l} 7 \\ 4 \\ 3 \end{array} \right.$	9	47	0	0
Male alcohol.....	6	7	10	66	4	6
Female alcohol.....	4	4	6	41	4	9.8
Double alcohol.....	$\left\{ \begin{array}{l} 6 \\ 2 \end{array} \right.$	$\left\{ \begin{array}{l} 4 \\ 9 \end{array} \right.$	11	73	5	6.8
Second generation alcohol.....	3	2½	3	25	1	4

As in my former experiments none of the control young died of lack of vitality. The alcohol lines show a small percentage of deaths—4 mice or six per cent. in the male alcohol line, 5 mice or 6.8 per cent. in the double alcohol line and 4 mice or 9.8 per cent. in the female alcohol line. The second generation alcohol lines had 1 death or 4 per cent. of all of their young. In the former experiments ('11) the fatalities were somewhat greater—9 young or 11.1 per cent. in the first generation of alcoholized mice and 7 young or 12.5 per cent. in the second generation. Stockard ('16), with his guinea pigs, had a fatality of 43 per cent. in the male alcohol line, 52 per cent. in the female alcohol line, 46 per cent. in the double alcohol line and 16 per cent. in the control line.

FECUNDITY

Table IX gives the average number of litters, average number of young, and average number in a litter for one female of the control line and one female of the male alcohol lines for seven months; for one female of the female alcohol line and double alcohol line for 4 months; and for the second generation alcohol line for 2½ months.

On account of the difference in the length of time the different lines were carried, it is impossible to make a direct comparison. However the greater fecundity of all the alcohol lines over the control line is striking. Though

TABLE IX
 FECUNDITY OF THE DIFFERENT LINES
Average of One Female of Each Line

Line	Months Observed	Average in a Litter	Average No. of Litters	Average No. of Young
Control.....	7	5.1	1.3	6.5
Male alcohol.....	7	6.6	1.66	11.0
Female alcohol.....	4	6.83	1.5	10.25
Double alcohol.....	4	6.5	1.4	9.3
Second generation alcohol.....	2½	8.3	1.0	8.3

the control mice were carried longer than any line except the male alcohol line, they have next to the lowest number of litters—the lowest being the second generation alcohol line carried only one third as long as they. They have the fewest young of all the lines and the smallest litters. The male alcohol line can be compared directly with the control line since they were both carried seven months. They have somewhat larger litters, somewhat greater average number of litters and nearly twice as many young as the controls. It is not possible to compare them directly with the lines that were only carried four months, but since the averages of these lines are almost as high as those of the male alcohol mice, it follows that the male alcohol mice were not as fecund as the female alcohol and double alcohol lines. The female alcohol mice show the greatest fecundity of all the lines, while the double alcohol and second generation of alcohol mice come next.

The three lines in which the females were alcoholized were somewhat more fecund than the line in which the males alone were alcoholized and decidedly more so than that in which neither parent was alcoholized. These results confirm those obtained in my former work ('11) where the control mice carried 7 months had 2.2 litters or 13.3 young on an average and the alcohol mice had 2.8 litters and 16.1 young; the second generation of control mice carried 4 months had 1.5 litters and 7.1 young, while the corresponding alcohol line had 1.8 litters or 12.4 young.

Why the mice had fewer young in these experiments

than in the former is not clear. It may have been due in part to the greater fluctuations of temperature in the laboratory building used here. Whatever the reason, the control mice in these experiments after the first few months occupied themselves in growing fat instead of having young.

Stockard's results on guinea pigs are directly contrary to these; his alcoholized animals had decidedly fewer young than the control guinea pigs.

COMPARISON OF THE GROWTH OF THE YOUNG IN THE VARIOUS LINES

In comparing the weights in Table X and Curve I it should be remembered that all of these young were alcoholized except the male alcohol line and of course the controls.

TABLE X
WEEKLY GROWTH OF THE YOUNG

Line	Average Weight of Female Parents	Average Weight of Male Parents	Number of Young Weighed	Average Weight per Mouse at Birth	Average Weight at One Week	Average Weight at Two Weeks	Average Weight at Three Weeks	Average Weight at Four Weeks	Average Weight at Five Weeks	Average Weight at Six Weeks	Average Weight at Seven Weeks	Average Weight at Eight Weeks
Controls.....	22.6	21.5	31	1.4	2.3	3.8	4.9	6.6	8.7	10.2	11.2	11.6
Male alcohol.....	23.2	24.8	25	1.2	2.1	3.7	4.8	7.4	9.3	11.1	13.1	13.6
Female alcohol.....	20.2	21.4	21	1.1	2.5	3.5	3.8	5.5	7.7	9.5	10.3	14.3
Double alcohol.....	21.1	23.7	13	1.2	2.6	3.5	5.9	8.4	9.0	10.9	11.3	13
Second generation alcohol...	13.5	17	11	1.1	2.4	3.8	4.7	6.4	8.8	11.5	14.5	15.1

The weights of all the lines at birth and for the first two weeks are quite similar. After that variations began. The young of the double alcohol line surpassed all for four and a half weeks, while the young of the female alcohol line fell behind all the others at the beginning of the third week and remained below up to the seventh week and at the eighth week they were next to the highest. The young of the control line, the male alcohol line and the second generation of the alcohol line grew at about the same rate up to the fifth week. At this time the weight of the male alcohol line slightly surpassed all the others; then the

second generation of alcohol mice outgrew all and continued ahead until the end of the experiment. After the sixth week there were rather wide variations and this continued as long as they were weighed. At the eighth week the weights of the different lines stood in the following order: second generation of alcohol mice 15.1 grams; female alcohol mice 14.3 grams; male alcohol mice 13.6 grams; the double alcohol mice 13 grams; and the controls 11.6 grams. In my former experiments ('11) the alcohol young surpassed the controls in the rate of growth.

COMPARISON OF THE DIFFERENT LINES

The control line had the fewest young of any of the lines; they had no deaths from lack of vitality; the growth of their young was slower than that of any of the other lines except the female alcohol line.

The male alcohol line was more fecund than the controls, but less so than the other alcohol lines; their death rate from lack of vitality was four mice or 6 per cent.; the growth of their young was better than that of the controls and female alcohol lines.

The female alcohol mice were the most fecund of all the lines; their death rate was four mice or 9.8 per cent.; their growth was even slower than the control mice until the last week, when they made a large gain and outgrew all but the second generation of alcohol mice.

The double alcohol mice were slightly less fecund than the female alcohol line; five mice or 6.8 per cent. of their young died; they grew a little faster than the controls.

For the second generation of alcohol mice, two males, offspring of the male alcohol line, were mated with two females, young of the same line and one female from the female alcohol line. Thus one grandmother and all but one of the grandfathers were alcoholized, the second generation were all alcoholized after they became adult and one from birth and their young also were alcoholized. The fecundity of the second generation of alcohol mice was high; they had one death from lack of vitality, or 4

per cent. of all their young, and their young grew the fastest of all the lines.

It is a matter of regret that owing to an accident—over-alcoholization one day—the second and third generations were not carried farther. However, as far as they went, no injurious effect from alcohol is apparent in fertility, nor vigor of growth, and but a small one in viability.

From indications in our results it would seem to be dangerous to draw far-reaching conclusions from data obtained on a single species. Although this work was not carried as long as it was planned, yet as it corroborates my former experiments in practically every detail it goes to prove that mice are to a degree resistant to alcohol whether it is fed or inhaled by them. From results obtained in bacteriological laboratories it is well known that mice are very resistant little animals, in comparison to sensitive animals like guinea pigs. Mice are immune to the toxin of the tetanus bacillus. It seems reasonable to expect that an animal which is immune to such a virulent toxin might have a considerable degree of resistance to the effects of alcohol.

SUMMARY

1. The white mice given alcohol by the inhalation method gave much the same results as those that received it in their food in my former experiments.

2. The fecundity of the alcohol mice was greater than that of the control mice, as in my former study.

3. Six per cent. of the young of the male alcohol line, 6.8 per cent. of the double alcohol line, 9.8 per cent. of the female alcohol line and 4 per cent. of the second generation alcohol line died from lowered vitality, while none of the control young died. Similar results were obtained in my former experiments, except that the alcohol line had a higher death rate—11.1 per cent. in the first generation and 12.5 per cent. in the second generation.

4. The growth of the young of all the alcohol lines ex-

ceeded that of the controls, as in my former experiments. The young of the second generation alcohol line outgrew all the others.

5. There were no abortions, no still births and no monsters obtained in these experiments, nor in the former.

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